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Effects of Heat and Ionizing Radiation on *Salmonella typhimurium* In Mechanically Deboned Chicken Meat

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ABSTRACT

Response-surface methodologies were used to examine the effects of gamma-irradiation temperature and dose preceded or followed by heating at 60°C for 3 min on the survival of *Salmonella typhimurium* in mechanically deboned chicken meat (MDCM). The effects of irradiation temperature and dose were significant. Heating the inoculated chicken meat before irradiation did not sensitize the bacteria to the effects of the ionizing radiation. Treating the inoculated chicken meat with gamma radiation made the *Salmonella* much more sensitive to the effects of heat. For example, 3 min of heat at 60°C followed by a radiation dose of 0.90 kGy at 0°C decreased the number of survivors by 6.4-log units; when the irradiation occurred prior to heating, the decrease was 8.9-log units. Independent studies revealed that the effect of cooking was directly dependent upon the irradiation dose regardless of the order in which the heat and radiation treatments were applied. The effect of irradiation on the survival of the *Salmonella* was not dependent on the amount of heat applied regardless of the order in which the treatments were applied. There was no evidence of a gamma-radiation, dose-dependent decrease in the thermal D_{10} value at 60°C of *S. typhimurium* in MDCM. The increased gamma-radiation, dose-dependent sensitivity of irradiated *Salmonella* in MDCM to heat did not change even when the irradiated meat was stored for periods of up to 6 weeks at 5°C prior to heating.

The treatment of poultry with ionizing radiation for the control of salmonellae and other foodborne pathogens was approved by the U.S. Food and Drug Administration on May 2, 1990 (*1*). It is estimated that 35% of chicken carcasses processed in the United States of America may be contaminated with *Salmonella* species (*3*). Treatment with ionizing radiation is an effective method for the elimination or marked reduction of salmonellae on poultry (*7,12,15,17,18,21,28,29*). Many of these authors also noted a significantly increased lethality for *Salmonella* with increased temperatures of irradiation. Incze et al. (*8*), Pallas and Hamdy (*20*), Schaffner et al. (*23*), Szczawinska (*26*), and Shamsuzzaman (*24*) proposed the simultaneous application of ionizing radiation and heat for the control of pathogens in foods. In part, these proposals make use of the well-known principle that reaction rates approximately double with each 10°C increase in temperature, but the actual relationship between irradiation temperature and lethality is more complicated (*4*). If such combination treatments, particularly as applied to less than sterilization levels, produce additive effects, then they may allow the use of smaller radiation doses. A decreased radiation dose would produce fewer deleterious effects on the organoleptic and micronutrient properties of the product and still in combination with heat adequately control foodborne pathogens, but the use of temperatures above those currently approved for the processing of poultry in the United States would require regulatory review and approval.

Investigators have reported synergistic effects on bacterial spores after separate irradiation and heat treatments (*6,9-11,14,16*). Okazawa and Matsuyama (*19*) observed that the simultaneous application of ionizing radiation and heat was more effective against *Escherichia coli* K12 in broth than were separate treatments. The growth phase of the cells was important. Szczawinska (*26*) irradiated *Staphylococcus aureus, Salmonella typhimurium, E. coli,* and *Pseudomonas fluorescens* in broth killing approximately 90% of the cells in each case, and then heated the cells at 70°C. *S. aureus, E. coli,* and *P. fluorescens* were much more sensitive to heat but *S. typhimurium* was not. Licciardello (*13*) reported that the sensitivity of *S. typhimurium* in egg yolk to gamma radiation increased as a function of irradiation temperature when previously irradiated cultures were heated at lethal temperatures (above 43°C).

Irradiated fresh poultry meat products will not generally be eaten raw or without additional heating if heating has been used during processing. It is important, therefore, to determine if a heat treatment applied before or after a treatment with ionizing radiation or after refrigerated storage of an irradiated product is equally effective as when applied immediately before or after irradiation.

In the following report mechanically deboned chicken

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meat (MDCM) is used as a model system because it is a commercial product and because it has been implicated in the transmission of *Salmonella*. The particle size of this product allows for relatively homogeneous replicate samples from a particular lot of commercially produced meat; furthermore, by irradiation at -40°C *in vacuo* to a dose of 42 kGy the product can be sterilized with little, if any, alteration of its physical or chemical properties (*30*). This eliminates the variable of competing natural flora from the study. The objective of this study was to examine the effects of irradiation temperature and dose preceded or followed by heating or refrigerated storage and heating on the survival of *S. typhimurium* on MDCM and to determine if radiation and heat act synergistically in this medium.

MATERIALS AND METHODS

Culture

Salmonella typhimurium ATCC 14028 was maintained and cloned on tryptic soy agar (TSA; Difco, Detroit, MI) and incubated at 35°C. One milliliter from a 15-18 h culture incubated at 35°C of *S. typhimurium* in trypticase soy broth (TSB; BBL, Cockeysville, MD) was used per 100 ml for inoculation of TSB in 500-ml baffled shake flasks. The cultures were incubated with shaking (150 rpm) for 16 h at 35°C. A 10-fold concentrated inoculum was prepared by centrifuging and resuspending the cells in one-tenth volume of 0.1% peptone water (Difco).

Mechanically deboned chicken meat

Mechanically deboned chicken meat in 18-kg lots consisting of approximately 90% rib and 10% back meat was obtained from a commercial manufacturer of poultry frankfurters. The proximate analysis of this product was 67.6% moisture, 17.7% fat, and 13.6% protein. The chicken meat was subdivided into 50.0 ± 0.05-g lots and then spread thinly and vacuum sealed in Stomacher 400 polyethylene bags. These bags were themselves vacuum sealed in American National Can Company Freshstuff oxygen barrier pouches (oxygen transmission 0.6-0.8 cc/645 cm^2/24 h at 3.5°C and 90% RH). These replicate samples of MDCM were then frozen and gamma irradiated at -40°C to an absorbed dose of 42 kGy and then stored at -20°C until used. Sterility was confirmed by plate count.

Sample inoculation and packaging

Each replicate of 150 g of sterile MDCM in a No. 400 Stomacher bag was inoculated with 15 ml of the 10 x cell concentrate of *S. typhimurium* ATCC 14028, described above, and mixed for 45 s using a Stomacher. This provided an inoculum of approximately 10^9 cells per g of meat. After mixing, 5.0 ± 0.05-g samples were removed aseptically and vacuum packaged in preweighed sterile 17.5 by 17.5 cm International Kenfield All-Vak Number 13 pouches (International Kenfield Distributing Co., Rosemont, IL). The pouch film has a low oxygen permeability (1.0 ml/645 cm^2/24 h), is suitable for boil-in applications and consists of nylon (2 ml) with a 1-ml food-contacting layer of medium density polyethylene. Both nylon and polyethylene are suitable for use with ionizing radiation (*27*). In subsequent studies ordinary No. 400 polyethylene Stomacher bags were adequate for heat treatments at temperatures of 60°C. These bags were themselves sealed in American National Can Company Freshstuff bags for storage or irradiation treatments.

The processing and irradiation conditions chosen for these studies were different from those that would ordinarily be used for poultry, i.e., the product was free from microorganisms except for the inoculum and the irradiation was performed *in vacuo*. Sterile MDCM was used to eliminate the variable of competing natural indigenous microflora; the high inoculum levels were used to allow adequate statistics to be obtained at all treatment levels; and the inoculated samples were irradiated *in vacuo* to eliminate the variable of differing amounts of oxygen on the survival of the inoculum.

Heating

Individual pouches of inoculated meat were heat treated by total immersion into an agitated water bath. Preliminary studies established that a bath temperature of 60°± 0.1°C allowed varied treatment times and enough remaining viable cells to determine the additional effects of irradiation. The 5.0-g samples reached 60°C within 15 s after submersion in the water. After heating the samples were cooled to 0°C in ice water.

Irradiation

Samples were gamma irradiated in a self-contained source (135,708 Ci, ^{137}Cs) producing a dose rate of 0.12 kGy per min. The dosimetry and dose distribution were described by Shieh et al. (*25*). Routine dosimetry was conducted with ferrous sulfate/cupric sulfate dosimeters. The samples were brought to temperature before irradiation, and this temperature was maintained ± 2°C during irradiation by injecting of the gas phase from liquid nitrogen. Because of the low heat capacity of gaseous nitrogen, the actual variation in sample temperature did not exceed 0.5°C. The sample bags were hung vertically in a uniform portion of the radiation field and arranged to minimize any differences in radiation dose. The fact that the samples were spread uniformly in a thin layer over a 10 by 10 cm area within each bag helped to ensure that the absorbed radiation dose was uniform.

Response-surface study

The following irradiation doses and temperatures were included in the response-surface design: 0 kGy (-20, 0, and 20°C); 0.45 kGy (-10 and 10°C); 0.90 kGy (-20, 0, and 20°C); 1.35 kGy (-10 and 10°C); and 1.80 kGy (-20, 0, and 20°C). Heat treatments were for 3.0 min at 60 ± 0.1°C. The following heating and irradiation sequences were investigated: irradiated, irradiated and then heated, and heated and then irradiated. Each treatment was replicated twice.

Varied radiation doses and constant heat treatment

Inoculated samples of meat were irradiated at 0°C to absorbed doses of 0, 0.45, 0.90, 1.35, and 1.80 kGy and either not heated, or heated for 1.5 min at 60°C before or after irradiation. The irradiation treatment was replicated four times. The heating then irradiation and the irradiation then heating treatments were each replicated twice.

Varied heating times and a constant radiation dose with and without storage

Inoculated samples of meat were heated at 60°C for 0, 1.0, 2.0, or 3.0 min and either not irradiated or irradiated to 0.90 kGy at 0°C before or after heating. Each treatment was replicated twice. In a separate study, inoculated samples of meat were heated at 60°C for 0, 0.5, 1.0, 1.5, 2.0, 2.5, or 3.0 min and irradiated to 0.90 kGy at 0°C before or after, heating. This experiment was replicated four times; two of the replicates were stored for 6 d at 5°C before the second treatment of heat or irradiation was applied.

Thermal D_{10} values for irradiated S. typhimurium

In two independent studies 200 g of sterile MDCM were inoculated with approximately $10^{9.9}$ *S. typhimurium* ATCC 14028 cells per g and mixed well with the meat by stomaching for 1.5

min. The inoculated MDCM was aseptically subdivided into 5.0 ± 0.05-g samples, placed into sterile No. 400 polyethylene Stomacher bags, spread uniformly over a 10 by 10 cm area, and vacuum sealed at approximately -0.99 bar. Samples were irradiated at each of the following absorbed gamma radiation doses: 0, 0.45, 0.90, 1.35, and 1.80 kGy at an irradiation temperature of 0°C. Following irradiation individual samples were heated at 60°C for 0, 15, 30, 45, 60, 90, and 120 s.

Varied radiation doses and storage times with and without a heat treatment

Sterile MDCM (300 g) was inoculated with approximately $10^{9.46}$ *S. typhimurium* ATCC 14028 cells per g and mixed well by stomaching for 1.5 min. The inoculated MDCM was subdivided into 5.0 ± 0.05-g samples, placed into sterile No. 400 polyethylene Stomacher bags, spread uniformly over a 10 by 10 cm area, and vacuum sealed at approximately -0.99 bar. All eight samples were irradiated to absorbed gamma radiation doses of 0, 0.75, 1.5, 2.25, and 3.0 kGy at an irradiation temperature of 0°C. All samples were then placed in refrigerated storage at 5°C. After the appropriate period of storage of 0, 14, 28, or 42 d, two samples were removed from storage for analysis and/or further treatment. One set was analyzed after irradiation and storage. The second set was heated in a well-mixed water bath by submersion for 2.0 min at a temperature of 60 ± 0.1°C and then rapidly cooled by submersion in an ice bath. The number of survivors was determined as described below. The entire study was replicated twice.

Microbiological assay

Samples were assayed for colony-forming units (CFU) by standard pour-plate procedures with serial dilutions in sterile 0.1% Difco bacto peptone water. The pour plates were prepared using TSA and incubated for 24 h at 35°C. The CFU on three petri plates at a dilution giving 30 to 300 colonies were counted with a New Brunswick Scientific Biotran II automated colony counter.

Statistical analysis

Responses were expressed as the logarithm of the number of CFU per g. These responses were converted into survival values, that is the logarithm_{10} of [the number of CFU (N) divided by the initial number of CFU (N_0)]. Regression techniques were used to fit second order response-surface models to the data to predict the number of survivors following a given treatment (2). Graphically, these results are presented as three dimensional survival curves where the logarithm of (N/N_0) is plotted against radiation dose and temperature. Using this format the destruction of one log of CFU ($1D_{10}$) has the value of -1.0, and D_{10} values are the negative reciprocal of the slope of the individual regression of the logarithm (N/N_0) plotted against radiation dose or, in the case of thermal D_{10} values, time.

Thermal D_{10} values were determined by least squares analysis using the REG procedure of the SAS system for linear regression using values from the linear portion of the survival curve. The N_0 values were not themselves used in the computation of the regressions to eliminate possible shoulder effects. At least four treatment times were used in the calculation of each regression. Individual regressions were compared by analysis of covariance. Statistical calculations were performed with the General Linear Models (GLM) procedure of the SAS statistical package *(5,22)*. Significance is reported at the 0.01 level. Because very high inocula were used for these studies, estimates of surviving CFU were generally obtained with three significant figures, and the results are expressed here with two significant figures.

RESULTS AND DISCUSSION

Response-surface study

The results obtained when MDCM is inoculated with *S. typhimurium* and irradiated, heated for 3 min at 60°C and then irradiated, and irradiated and then heated for 3 min at 60°C are presented in Table 1. The effects of radiation dose on the survival of *S. typhimurium* in the MDCM were significant for each of the three treatments. There were significant effects due to irradiation temperature and interaction between radiation dose and temperature in the irradiated samples and the irradiated samples with a prior heat treatment. The effect of irradiation temperature was not

TABLE 1. S. typhimurium *ATCC 14028 colony-forming units (CFU) per gram of mechanically deboned chicken meat after treatment with gamma radiation, heat for 3 min at 60°C and then gamma radiation, or gamma radiation and then heat for 3 min at 60°C.*

Dose kGy	Irradiation temperature C	Irradiated	Heated then irradiated	Irradiated then heated
		Logarithm CFU per gram ± S. D.		
		N = 4	N = 2	N = 2
0	-20	9.75 ± 0.11	6.04 ± 0.45	5.67 ± 0.16
0.90	-20	8.77 ± 0.13	4.95 ± 0.35	1.59 ± 0.16
1.80	-20	7.02 ± 0.48	3.51 ± 0.38	0.72 ± 0.17
0.45	-10	8.90 ± 0.16	4.79 ± 0	2.17 ± 0.19
1.35	-10	7.73 ± 0.60	3.22 ± 0.10	0.00 ± 0
0	0	9.73 ± 0.12	5.79 ± 0.06	4.29 ± 2.15
0.90	0	8.20 ± 0.10	3.38 ± 0.06	0.84 ± 0
1.80	0	6.05 ± 0.07	1.34 ± 0.46	0.00 ± 0
0.45	+10	8.80 ± 0.15	4.08 ± 0.04	2.12 ± 0.13
1.35	+10	7.10 ± 0.12	2.31 ± 0.25	0.00 ± 0
0	+20	9.75 ± 0.08	5.23 ± 0.06	5.80 ± 0.04
0.90	+20	8.04 ± 0.08	2.78 ± 0.07	0.00 ± 0
1.80	+20	5.49 ± 0.10	0.00 ± 0	0.00 ± 0

TABLE 2. *Response-surface equations predicting the effects of gamma irradiation, heating for 3 min at 60°C followed by gamma irradiation, and gamma irradiation followed by heating for 3 min at 60°C at irradiation temperatures of -20 to +20°C on the survival of* S. typhimurium *ATCC 14028 on mechanically deboned chicken meat.*

Irradiated

Log_{10} survivors = -0.1637 + 0.0009 x TEMP - 1.1359 x DOSE - 0.0219 x TEMP x DOSE + 0.0004 x $TEMP^2$ - 0.4359 x $DOSE^2$

R-square = 0.980

Heated then irradiated

Log_{10} survivors = -4.2252 - 0.0225 x TEMP - 2.1861 x DOSE - 0.0281 x TEMP x DOSE + 0.0008 x $TEMP^2$ + 0.0436 x $DOSE^2$

R-square = 0.976

Irradiated then heated

Log_{10} survivors = -5.0121 - 0.0059 x TEMP - 7.0121 x DOSE - 0.0110 x TEMP x DOSE + 0.0014 x $TEMP^2$ + 2.3798 x $DOSE^2$

R-square = 0.932

Survivors = number of CFU surviving a given treatment divided by the number of CFU in the untreated samples. TEMP = Degrees Centigrade. DOSE = gamma radiation dose in kGy.

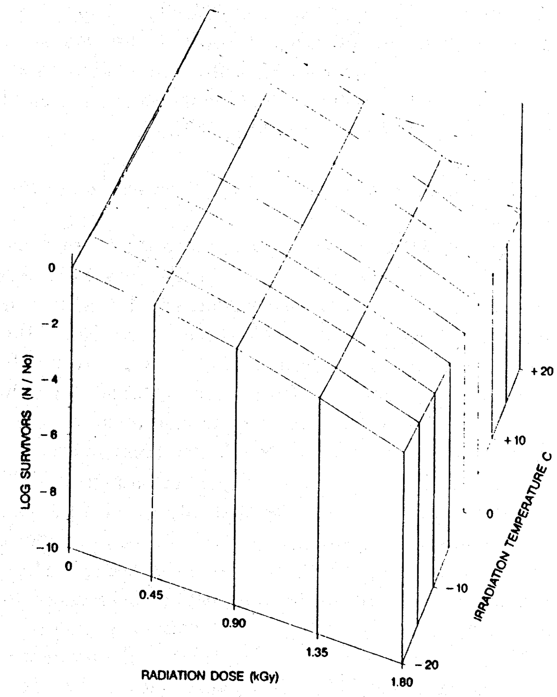

Figure 1. *Predicted response of* S. typhimurium *to treatment with gamma radiation in vacuum-packaged, mechanically deboned chicken meat at irradiation temperatures of -20 to +20°C and absorbed radiation doses of 0 to 1.80 kGy.*

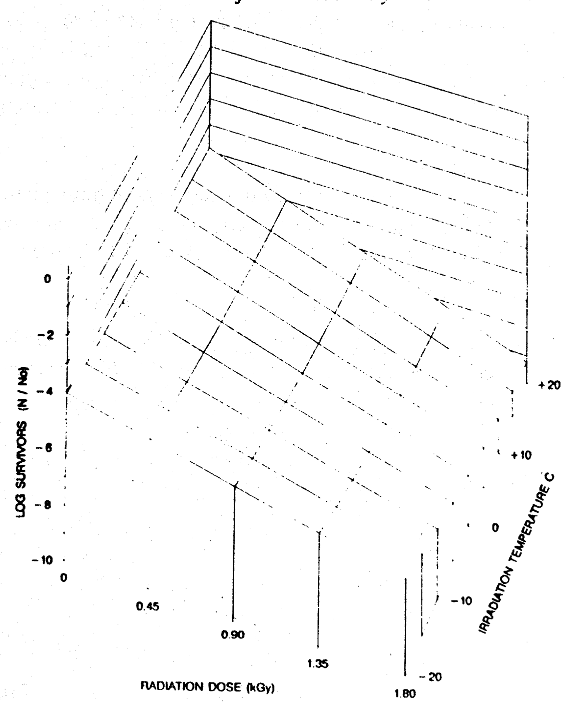

Figure 2. *Predicted response of* S. typhimurium *to heating for 3.0 min at 60°C followed by treatment with gamma radiation in vacuum-packaged, mechanically deboned chicken meat at irradiation temperatures of -20 to +20°C and absorbed radiation doses of 0 to 1.80 kGy.*

significant in samples heated after irradiation. The number of surviving *S. typhimurium* CFU was decreased by irradiation to an absorbed dose of 0.90 kGy administered at 0°C by 1.54 logs, by heating the inoculated MDCM for 3 min at 60°C by 4.27 logs, by heating and then irradiating to 0.90 kGy at 0°C by 6.40 logs, and by irradiating to 0.90 kGy at 0°C and then heating by 8.9 logs. [The effects of heating alone were determined by subtracting the mean of the six values for the nonirradiated heated samples (5.47) from the mean of the three nonradiated samples (9.74).] If the effects of irradiation and heating were strictly additive, then irradiation to a dose of 0.90 kGy at 0°C plus heating either before or after irradiation would be expected to decrease the CFU by approximately 5.8 logs; and, in fact, when heating occurred before irradiation the results were not very different from those of the prediction. But when the irradiation treatment preceded the heat treatment, the decrease in CFU of surviving *S. typhimurium* was much greater than expected. The predictive equations derived from these data are reported in Table 2. They allow consideration of the effects of irradiation dose and temperature and prediction of the effects of heating prior to or following irradiation treatment of the MDCM, which contain *S. typhimurium*. The predictions of the three equations agreed closely with the actual results and are presented graphically in Fig. 1, 2, and 3. The respective R-square values give an indication of the fit of the equations to the real data. The predictions of the responses of *S. typhimurium* to gamma irradiation or gamma irradiation followed by this very mild heating treatment are striking in their implications. The USDA Food Safety and Inspection Service requested in their petition a minimum dose of 1.5 kGy and a maximum dose of 3.0 kGy, however, the current regulation (*1*) establishes only a maximum dose of 3.0 kGy for the control of *Salmonella* in poultry. These equations predict that a radiation dose of 1.5 kGy would kill 2.04, 2.49, 2.85, 3.13, or 3.33 log CFU units of *S. typhimurium* at irradiation temperatures of -20, -10, 0, + 10, or +20°C, respectively. The same radiation dose applied at the same irradiation temperatures followed by heating for 3 min at 60°C would kill 9.17, 9.82, 10.18, 10.26, or 10.06 log CFU units of *S. typhimurium*. The results would be almost

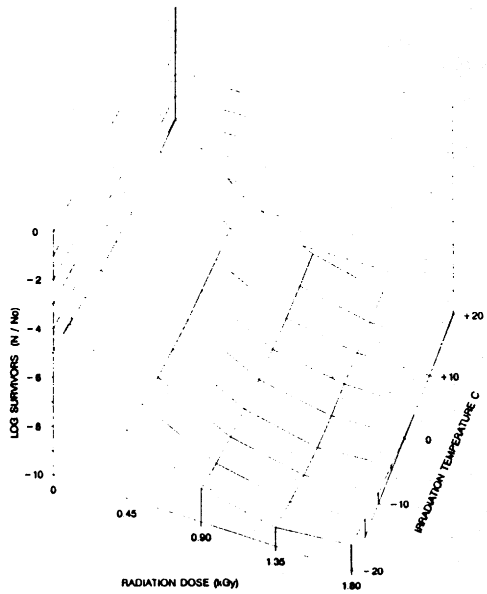

Figure 3. *Predicted response of* S. typhimurium *to treatment with gamma radiation followed by heating for 3.0 min at 60 °C in vacuum-packaged, mechanically deboned chicken meat at irradiation temperatures of -20 to +20°C and absorbed radiation doses of 0 to 1.80 kGy.*

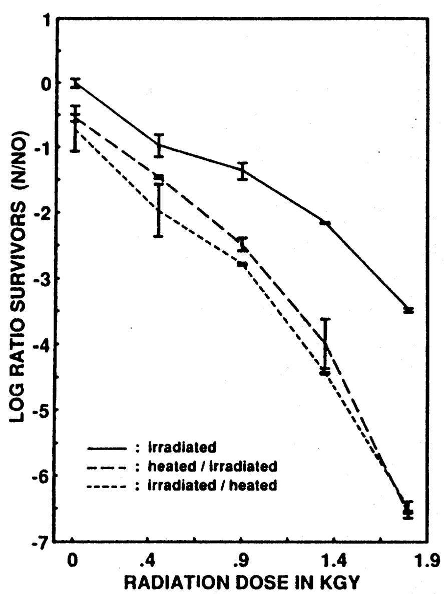

Figure 4. *Response of* S. typhimurium *in vacuum-packaged, mechanically deboned chicken meat to treatment with gamma radiation at 0°C, to treatment by heating for 1.5 min at 60°C followed by treatment with gamma radiation at 0°C, and to treatment with gamma radiation followed by heating for 1.5 min at 60°C.*

identical at a radiation dose of 0.90 kGy. The results indicate that chicken that has been irradiated and then cooked, even mildly, will have a greatly reduced probability for the survival of any *Salmonella* cells.

Varied radiation doses and constant heat treatment

This study was specifically conducted to test the following hypothesis: If the effects of irradiation and heating are simply additive, then treating the inoculated samples with various doses of radiation and the same heat treatment should generate parallel survivor curves regardless of the order in which the treatments are given. Because heating for 3 min substantially decreased the number of survivors in the previous response-surface study, the heating treatment was decreased to 1.5 min at 60°C in this study. This allowed the observation of the effects of the various radiation doses plus the mild heading over a greater range of radiation doses. The results are presented graphically in Fig. 4. The separation between the irradiation survivor curve and the other survival curves increased with increased radiation dose. Analysis of variance indicated significant effects for treatment, dose, and dose-treatment interaction; and analyses of covariances indicated that the regressions for the $\log_{10}$ of the surviving *S. typhimurium* in irradiated MDCM differed significantly from the regressions for the surviving *S. typhimurium* in MDCM, which had also been heated. There was, also, a significant interaction between the regressions and radiation dose. An analysis of covariance did not, however, indicate a significant difference between the regressions of the $\log_{10}$ of the surviving *S. typhimurium* in irradiated and then heated MDCM or heated and then irradiated MDCM. The significant dose-treatment interaction supports a conclusion that the magnitude of the radiation dose influenced the extent of the reaction of the cells to heat. A similar dose-treatment interaction was reported by Licciardello when *S. typhimurium* was irradiated and then heated in egg yolk (*13*).

Varied heating times and a constant radiation dose with and without storage

This study was designed to test this hypothesis: If the effects of irradiation and heating are simply additive, then treating the inoculated samples with various doses of heat and the same radiation treatment should generate parallel survivor curves regardless of the treatment order. The

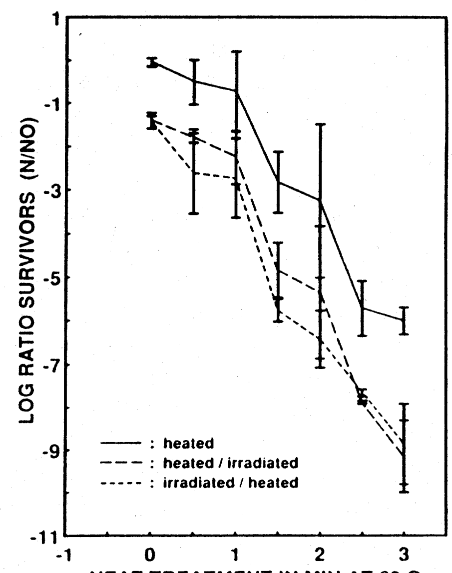

Figure 5. *Response of* S. typhimurium *in vacuum-packaged, mechanically deboned chicken meat to treatment by heating at 60°C, to treatment by heating at 60°C followed by treatment with a gamma radiation dose of 0.90 kGy at 0°C, and to treatment with a gamma radiation dose of 0.90 kGy at 0°C followed by heating at 60°C.*

TABLE 3. *Thermal D-values for* S. typhimurium *ATCC 14028 on mechanically deboned chicken meat which has been irradiated prior to heating at 60°C.*

Radiation dose (kGy)	N	D_{10}-value (s)	Standard error
0	8	17.4	2.8
0.45	8	19.2	2.6
0.90	8	17.4	1.8
1.35	8	13.8	0.7
1.80	6	13.8	1.9

results of this study are presented graphically in Fig. 5. When the cooking treatment was changed and the radiation dose remained constant at 0.90 kGy, the separation between the heat survivor curve for *S. typhimurium* from those of the heated and irradiated or irradiated and heated survivor curves increased only slightly with increased heat treatment. Analyses of variances revealed significant effects for treatment and heating time but not for the interaction between treatment and heating time. Analysis of covariances revealed significant differences between the regressions for the heated samples and for those that were also irradiated. The regressions for samples that were irradiated and then heated and for those that were heated and then irradiated did not differ significantly. The interactions between minutes of heating and the regression were not significant. Storing the samples for 6 d at 5°C before applying the second treatment of either heat or gamma radiation did not alter the results from those obtained at day zero. These results indicate that the heating period at 60°C does not influence the effect of a given radiation dose. These results agree with those reported by Kempe (*10*) that heat shocking spores of *Clostridium botulinum* did not sensitize the spores to gamma radiation, but that preirradition did sensitize the spores to heat. Gombas and Gomez (*6*) reported similar results with sensitizing spores of *Clostridium perfringens* to heat by preirradiation.

Thermal D_{10} values for irradiated S. typhimurium

This study tested the hypothesis that prior treatment with ionizing radiation does not alter the thermal D_{10} value for *S. typhimurium* in MDCM. A radiation dose-dependent decrease in the thermal D_{10} value of *S. typhimurium* would be considered as evidence for synergy. The results of this study are presented in Table 3. Not one of the D_{10} values presented in Table 3 differed significantly from any other as revealed by analysis of covariance of the regressions from which they were calculated. It is possible that the effects of the MDCM on both the radiation and thermal D_{10} values mask a positive effect. Nevertheless, these data do not support the rejection of the null hypothesis.

Varied radiation doses and storage times with and without a heat treatment

This study tested the hypothesis that any increased lethality for *S. typhimurium* as a result of irradiating the contaminated chicken before a heat treatment would disappear upon storage of the irradiated samples at a proper refrigeration temperature (5°C) because the radiation injury to the cells would be cured during refrigerated storage. The results of the 6-week study of the survival of S. *typhimurium* in irradiated MDCM or irradiated MDCM that was heated 2 min at 60°C after storage at 5°C are presented in Fig. 6. Analysis of variance for each regression revealed significant effects of radiation dose and heat but insignificant effects of storage time. Furthermore, the radiation dose-dependent effect of heating following irradiation did not diminish even when the irradiated samples were stored for as long as 6 weeks before heating (Fig. 6). This was confirmed statistically by computing a regression for the difference between the irradiated and the irradiated and heated log-survivor values at 0, 0.75, and 1.5 kGy. Higher doses were not considered because essentially all CFU had been inactivated by a radiation dose of 1.5 kGy combined with the heat treatment. Again the analysis of variance indicated a significant effect for dose but not for storage time. Thus, the effects of heat on irradiated *S. typhimurium* will depend upon the radiation dose to which it is exposed, and this effect will not decrease during refrigerated storage. The authors conclude that irradiated poultry will be much safer for the consumer than expected because the irradiation treatment will have made any surviving cells of *Salmonella* more sensitive to heat.

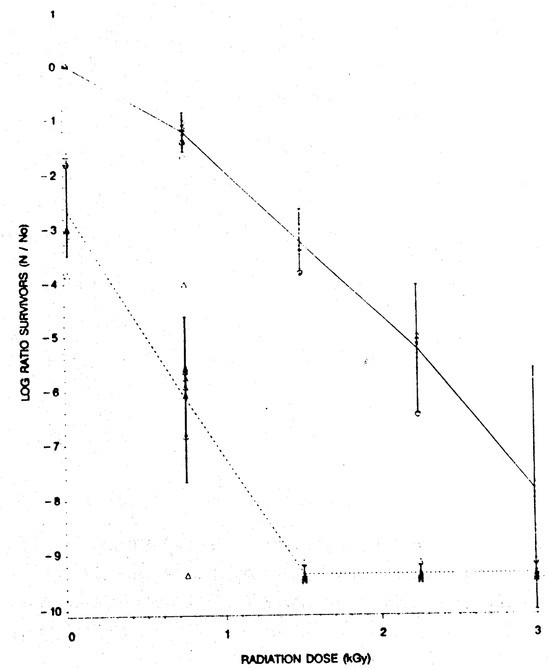

Figure 6. *Response of* S. typhimurium *in vacuum-packaged, mechanically deboned chicken meat to treatment with gamma radiaiton at 0°C (____) or to treatment with gamma radiation at 0°C followed by heating at 60°C for 2.0 min (---). Samples were heated and analyzed after 0, 2, 4, and 6 weeks of storage at 5°C. The vertical bar stands for the geometric mean ± SD.*

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